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# SEXUAL REPRODUCTION IN THE RUSTS.

A. H. CHRISTMAN.

(WITH PLATE VIII)

THE nature of the aecidium has remained one of the most vexed questions in mycology. BLACKMAN'S[1] recent discovery goes far toward clearing up the most important points and my own results, described below, confirm his general conclusions, though showing a widely different method of conjugation. The literature relating to sexuality and the general cell processes in the rusts has been so thoroughly and justly reviewed and critically estimated by BLACKMAN that I need hardly devote further space to it here.

My studies on the winter conditions of the rusts and the nature of the aecidium led me to the preparation of material for cytological study, and in the spring of 1904 Professor J. C. ARTHUR proposed to Professor HARPER to furnish material from his cultures for cytological investigation. This material was turned over to me. It consisted of a quantity of carefully identified aecidia of several rusts, and since our knowledge of the method of origin of the binucleated condition found in the aecidiospores was at that time entirely lacking as to the details of the behavior of both cells and nuclei, my study was directed largely toward that point.

It was soon found that aecidia of the caeoma type, with unlimited growth, were by far the most favorable material. This type of development apparently admits of less crowding of the hyphae, and as a result the cells and their relations can be made out more easily. Two forms were further found especially favorable because of the large size of their cells and nuclei. These were *Caeoma nitens* S. growing on *Rubus* cult., and *Phragmidium speciosum* Fr. on *Rosa humilis*.

The best fixation was secured by the use of Flemming's fixing solutions; both the strong and weak were very satisfactory. Fixing solutions were sent to Professor ARTHUR and the specimens were put into them by him at Lafayette, Indiana, and mailed at once to Madison. The usual time for fixation was from two to three days. Flemming's triple stain gave the best results. Good preparations were also obtained by the use of Heidenhain's haematoxylin.

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[1] BLACKMAN, V. H., On the fertilization, alternation of generations, and general cytology of the Uredineae. Annals of Botany **18**:323–373. *pls. 21–24.* 1904.

In the case of Phragmidium, material was found of all ages, so that the entire history from the uninucleated mycelial cells to the fully formed binucleated spores could be easily followed. It was found that in the young pustule the hyphae form a layer just beneath the epidermal cells (*fig. 1*). This layer, in which the direction of the individual hyphae is almost lost, is usually from one to three cells thick. The mycelial cells here are much thicker and shorter than those found in the vegetative hyphae. Each cell has a very dense, finely granular cytoplasm and a single large nucleus. The nucleus occupies a central position in the cell. There is a well-defined nuclear membrane and one large nucleole. The chromatin is always more or less massed at this stage, making a ragged net with the strands very irregular in thickness. Thus a great deal of clear space is left in the nucleus, which is filled with nuclear sap. The chromatin is regularly stained by the violet of the triple stain and the nucleole stains a deep red.

Certain cells now become elongated in a direction perpendicular to the epidermis, forming a continuous series and raising the epidermis (*fig. 2*). The exact origin of the cells of this series is hard to determine; many of them can be seen to be end cells of short hyphal branches. In thick sections, long branches may often be traced between the subepidermal cells of the host, which terminate in one of these cells. In the caeoma the cells are not crowded; there is even considerable intercellular space. Very often it may be seen that cells standing side by side arise from different hyphal branches, or at least from distant parts of the same hypha (*fig. 2*, *a* and *b*).

The single nucleus of the cell now apparently divides and the cell elongates into a rather narrower upper part which is cut off as a small distal cell from the larger basal cell. The cell division is very unequal. The end cell as a result is only about one-third the size of the cell beneath it (*fig. 3*). The protoplasm of the end cell is at first dense and appears quite normal. It soon becomes very vacuolar, however, and finally appears quite clear, with only a few strands of granular material, and the whole cell dwindles in size. The chromatin of the nucleus seems never to have passed into the resting condition, but lies in dense masses, apparently as it was left on reaching the poles of the spindle in the nuclear division preceding. The whole nucleus is very small compared with the nucleus of the larger cell beneath and contains little space between the chromatin masses. The material of the nucleus begins to disorganize and soon becomes a homogeneous mass, which stains a hazy red with the triple stain.

The cell beneath this terminal cell, on the other hand, begins to enlarge, bulging at the middle, and becomes irregularly barrel-shaped. The cytoplasm is dense with small vacuoles. The nucleus enlarges greatly and the chromatin matter is distributed through its interior, forming a ragged network. There is a large, well-defined nucleole.

We have thus a series of oblong cells standing vertically side by side but not much crowded. If we study a series from the margin of a sorus to its center, we find gradually more advanced stages, so that very many conditions of development may often be found in a single section.

Up to this time in the history, the writer's observations agree very closely with those of BLACKMAN. The subsequent behavior of the larger cells, however, which BLACKMAN terms the fertile cells, is very different. Many times two can be found which incline toward each other, coming in contact in a region on their adjacent walls. At this period the remains of the degenerating sterile cells may often still be seen (*fig. 4*). At the point of contact an opening is formed by solution of the cell wall and thus the protoplasts are brought into contact. The pore is small at first, leaving the bases of the gametes quite independent, and often the two tips of the gametes are also separate, as is indicated by the notch at the apex of the conjugated cells (*fig. 5*). By gradual enlargement of the pore the upper halves of the protoplasts of the gametes unite to form a continuous cell mass which still shows plainly the two distinct bases (*fig. 6*). The nuclei, which before fusion occupied a central position in the fertile cells, now come to lie in their upper portions, consequently the two nuclei are brought side by side in the conjugated region. Simultaneous division now takes place. Two spindles are formed which lie side by side in about the position in which the pore first appeared (*fig. 7*). The spindles in this division are more distinct and not quite so parallel as in subsequent conjugate divisions. Each shows a few short polar rays and to each pole are drawn several (certainly more than two, though the number was not determined) distinct chromosomes. The two nucleoli are at this time lying in the cytoplasm outside the spindle figures. They are less dense than at earlier stages and appear to be disintegrating. Two of the daughter nuclei, one from each spindle, wander back into the bases of their respective cells. The other two remain lying side by side and move into the distal end of the fused portion of the gametes, which now enlarges and elongates (*fig. 8*). A cell wall now cuts off the distal portion of this region, which contains the upper pair of nuclei, and thus the first aecidiospore mother-cell is formed (*fig. 9*). This cell, as has been described frequently, at once divides into two unequal cells, the aecidiospore and the small intercalary cell.

After one spore has been formed, the nuclei in the bases of the fused gametes again move upward into the conjugated portion, which has meanwhile grown in length, and the process of division is repeated. In this way a single row of aecidiospores is formed from each pair of the gametes (*fig. 10*). During these processes the cytoplasm retains the same granular appearance. The nuclei, excepting in figures showing division, always have the same general structure.

From the fact that the bases of the conjugating cells diverge widely, it is suggested that the conditions shown in *fig. 6* might be produced by the bending of a single hypha, as is the case in the development of an ascus. The earlier development of the gametes, however, shows conclusively that nothing of the sort occurs here, and that the cells which fuse may belong to distinct hyphal branches.

We find thus a typical case of the fusion of gametes at the base of each row of aecidiospores, with the difference that the nuclei do not fuse and the cell produced by the fusion germinates at once. The subsequent history of Phragmidium has been well worked out and I shall not take it up here.

My material of *Caeoma nitens* did not afford so complete a series of stages, but I have been able to trace with perfect certainty the history as far as the formation of the fertile and sterile cells. As in

Phragmidium, hyphae made up of large dense cells collect in layers one to four cells deep just beneath the epidermis of the host (*fig. 11*). From these hyphae a series of large elongated cells is formed which raise the epidermis. A cell wall cuts each of these cells into a sterile cell and the gamete (*fig. 12*).

The remainder of my material of Caeoma was so far advanced in development that each pair of gametes had already formed a considerable series of spores. Here, as in Phragmidium, a most conspicuous structure through all the later stages of the development of the aecidium is the basal remnant of the walls which separate the two gametes (see *figs. 6–10*). There may be more or less of it according to the completeness with which the adjacent walls were dissolved away when conjugation occurred, but in all cases it and the two distinct bases remain throughout as an evidence of the double origin of each row of aecidiospores (*fig. 10*).

Modifications of *figs. 6–10* sometimes occur when two gametes lie, before fusion, with the entire length of their adjacent walls in contact. A very complete destruction of the walls in the formation of the conjugation pore now may cause the two separate bases of the gametes almost to disappear. A section of *fig. 8*, showing a lateral or edge view, would appear as a single large cell containing two nuclei. Either of these conditions gives us a figure which might readily be mistaken for a binucleated basidium.

In *Uromyces Caladii* Pers. a further interesting modification was observed. In this form the fused portion of the gametes elongates greatly and the two nuclei come to lie in the upper part of this region. Much of the base of this region, and the two basal parts of the gametes, are occupied by a large vacuole (*fig. 13*). By normal conjugate division of the nuclei followed by cell division, an aecidiospore mother-cell is formed. The fused portion of the cell is so long and the base is so difficult to trace on account of the vacuole that this also may be readily mistaken for an ordinary binucleated cell.

Blackman considers the process described by him for *Phragmidium violaceum* as a "vegetative fertilization," in which an egg—the fertile cell—is fertilized by a vegetative nucleus, and his conclusions seem justified by the nature of the process in *Phragmidium violaceum*. In *P. speciosum* and *Caeoma nitens*, however, the cells which fuse are apparently equal gametes. If we consider the matter simply from this standpoint, the whole sorus must be considered as a collection of such pairs of equal gametes. The fusion of each pair should form a zygospore like that of the lower molds. There are, however, two important differences. The product of the fusion in the aecidium is a temporary structure which germinates at once, and further, in germinating it produces a considerable number of spores, the aecidiospores, while in the mold normally only one zygospore is produced. If it is found that the same method of fusion occurs in Micropuccinia this form might be considered an intermediate stage between the molds and Eupuccinia.

Further, it seems plain that each pair of gametes with the spores produced by them constitutes an individual structure. The aecidium cup, which has so often been considered the individual fruit body on account of its likeness to the cystocarp or pyrenocarp, is shown, by the discoveries described above, as well as by those of BLACKMAN, to be a complex of coordinate units rather than itself a morphological unit. An ascocarp is the product of the fusion of a single pair of gametes, while the aecidium arises from a collection of many such fusing pairs. Further, the peridium of an aecidium cup is not to be compared to the perithecial wall of the ascocarp, since it is composed of rows of cells which are morphologically equivalent to the aecidiospores. The tissue protecting the asci, on the other hand, is produced by the massing together of vegetative filaments which do not arise from the fertilized egg.

I am convinced that the method of conjugation above described will be found in the forms with the limited cup as well as in the more indefinite aecidial sori of Caeoma. Considering the aecidium a collection of individuals, it would seem that those forms having a peridium are to be regarded as the more highly specialized, while the caeoma is the more primitive type.

Against these views, which seem to me the obvious conclusions to be drawn from my discoveries, it may be urged that the spermogonia with their spermatia, showing as BLACKMAN points out very many resemblances to the male cells of the lichens and the red algae, are still left unexplained, and it must be admitted that there is ground for BLACKMAN'S suggestion of the origin of the rusts from the red algae.

RICHARDS'[2] observations of acarpogonial branch in the young aecidium of *U. Caladii* and other forms favor this view. It is quite possible that the gametes in *U. Caladii* are borne on branches arising from such a carpogonium. The final settlement of these questions of affinity must await the study of further forms of the rusts and related fungi. It is especially important, as suggested above, that the nuclear phenomena in species of rust with an abridged life cycle be determined. There can be no doubt that the proof given above of the existence of a sexual fusion of gametes in the aecidium finally disposes of the conception of DANGEARD[3] and SAPPIN-TROUFFY[4] that the teleutospore is an egg, and the nuclear fusion which occurs in it the equivalent of the sexual fusions elsewhere among the algae and fungi. On the other hand, these discoveries also confirm and give new significance to the conceptions, so clearly set forth by ARTHUR,[5] that the aecidium represents the stage of sexual rejuvenescence. There can be no question that BLACKMAN is in general correct in the contention that the nuclear phenomena in the teleutospore are concerned with chromosome reduction and mark the close of a sporophyte generation which began with the cell fusion in the aecidium. Here again, we need further facts as to the rusts with reduced life cycle before the doctrine of an alternation of generations, such as BLACKMAN maintains, can be regarded as finally established.

MAIRE'S[6] conception that the nuclear fusion in the teleutospore is a *mixie*, was developed on the basis of the belief that no real cell fusion occurs in the life cycle of the rusts. It is at least a fair presumption that while no nuclear fusion occurs in the aecidium, the fusion of gamete cells described above presents all the essential features of sexual conjugations as found in other plants and animals. Superficially considered, RACIBORSKI'S[7] conception that the sexual union may be regarded as consisting of two phases, cell fusion and nuclear fusion, might seem to fit the conditions found in the rusts. I am inclined, however, to accept BLACKMAN'S conclusion that the fusion in the teleutospore has wholly to do with the reduction of the number of chromosomes.

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[2] RICHARDS, H. M., On some points in the development of the aecidia. Proc. Amer. Acad. **31**:255–270. *pl. 1.* 1896.

[3] DANGEARD, P. A., La réproduction sexuelle des champignons. Le Botaniste, **7**:89–130. 1900.

[4] SAPPIN-TROUFFY, M., Recherches histologiques sur la famille des Urédinées. Le Botaniste **5**:59–244. *figs. 69.* 1896–97.

[5] ARTHUR, J. C., The aecidium as a device to restore vigor to the fungus. Proc. Soc. Promotion Agric. Sci., 23d annual meeting.

[6] MAIRE, R., Recherches cytologiques et taxonomiques sur les Basidiomycetes. 1902.

The difference between the method of conjugation in *P. violaceum* as described by BLACKMAN and *P. speciosum* as I have described it above indicates the necessity for a comparative study of a large number of aecidia to determine the nature of the sexual process in the group as a whole. There can be no question, however, that with the discoveries already made the existence of true sexual cell fusions in the rusts is finally established.

The writer feels greatly indebted to Professor ARTHUR for the careful selection and fixation of the material used in this work, and to Professor HARPER for valuable suggestions and kindly assistance in many ways.

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## EXPLANATION OF PLATE VIII.

The figures were drawn with the camera lucida. *Figs. 1, 2, 10, 11,* and *12* were drawn using the Bausch & Lomb 1-inch eyepiece and $\frac{1}{12}$ oil objective; all others were drawn with $\frac{1}{2}$-inch eyepiece and $\frac{1}{12}$ objective, the tube length in either case being $185^{mm}$.

### *Phragmidium speciosum.*

FIG. 1. Hyphae massing beneath the epidermis in the early stages of the caeoma.

FIG. 2. The series of large cells formed preparatory to division into sterile cell and gamete; *a* and *b*, cells lying close together, which appear to be parts of different hyphal branches.

FIG. 3. A cell of such a series as is shown in *fig. 2* after the division into the smaller sterile cell and the large gamete.

FIG. 4. Two gametes about to fuse; sterile cells remain though very much reduced in size.

FIG. 5. Fusing cells with the small conjugation pore just formed.

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[7] RACIBORSKI M., Über den Einfluss äusserer Bedingungen auf die Wachstumsweise des *Basidiobolus ranarum*. Flora **82**:107–132. *figs. 11.* 1896.

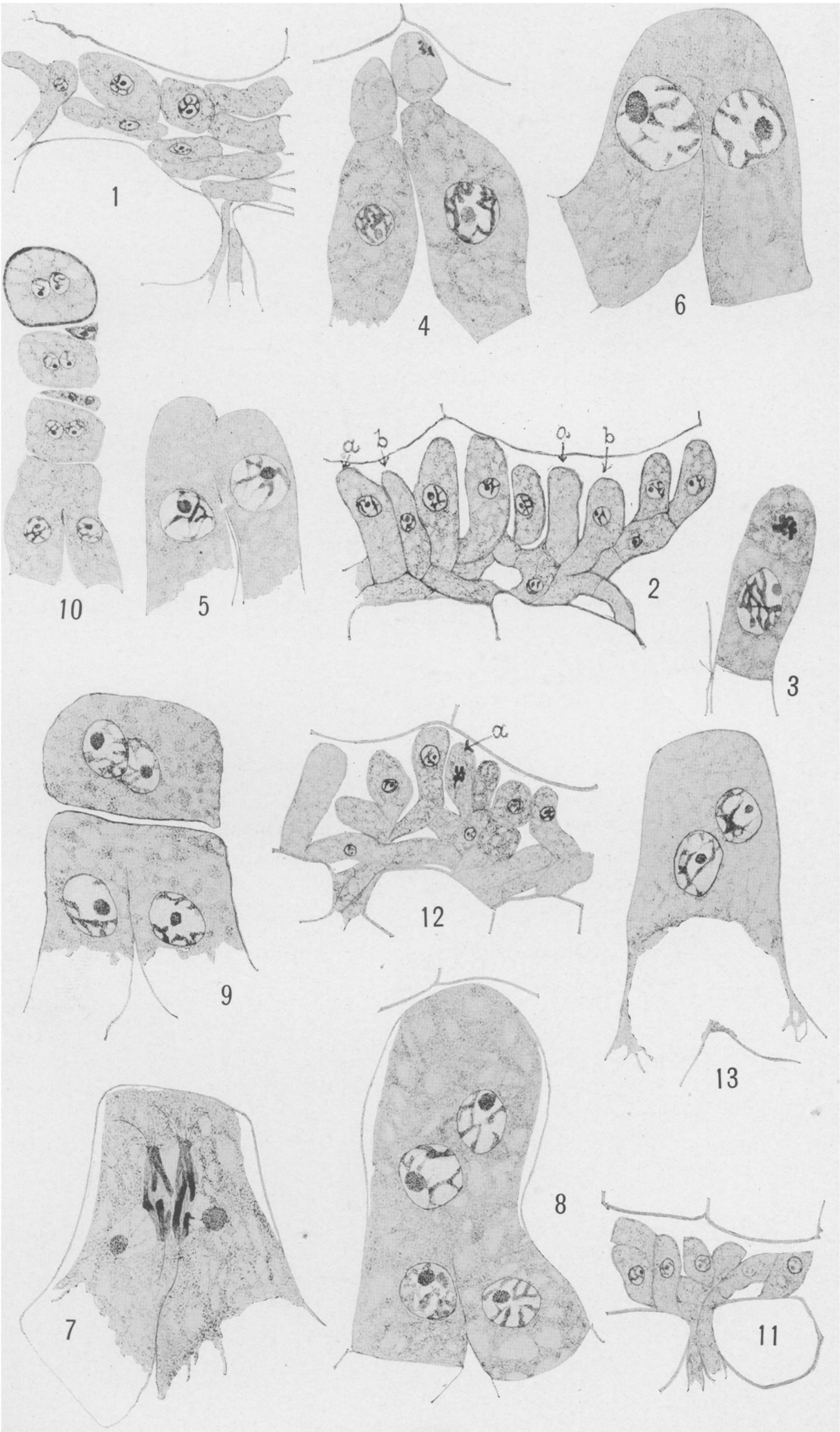

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FIG. 6. Same as *fig. 5*, slightly more advanced; the nuclei lie nearer the distal end than in earlier stages.

FIG. 7. Simultaneous nuclear division following the fusion.

FIG. 8. A stage following nuclear division in which the fused mass contains four nuclei, two side by side in the distal end and one in each of the bases.

FIG. 9. The aecidiospore mother-cell formed by a cell wall cutting off the upper portion of the protoplast.

FIG. 10. A figure taken from a thick section showing several aecidiospores, intercalary cells, and the two gametes at the base; the remnant of the basal part of the dividing wall being evident between the two gametes.

*Caeoma nitens.*

FIG. 11. Hyphae of rust massing beneath the epidermis in very young sorus.

FIG. 12. Series of large upright cells; *a*, a nuclear division preceding the throwing off of the sterile cell.

*Uromyces Caladii.*

FIG. 13. A pair of gametes fused, showing the nuclei in the upper portion of the protoplast, the large vacuole in the lower part, and the two less conspicuous bases.